**Spirulina Medium (modified)**

Aiba and Ogawa 1977, Schlösser 1994

First prepare the necessary stock solutions. Next, prepare Solutions I and II; Solution II includes 1 mL of the trace metals stock solution but not the vitamin stock. Autoclave Solutions I and II separately and cool; aseptically combine the two solutions. Aseptically add 1 mL of the cyanocobalamin (B\textsubscript{12}) solution.

<table>
<thead>
<tr>
<th>Component</th>
<th>Stock Solution</th>
<th>Quantity</th>
<th>Molar Concentration in Final Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Solution I</strong></td>
<td>500 mL</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>NaHCO\textsubscript{3}</td>
<td>---</td>
<td>13.61 g</td>
<td>1.62 x 10\textsuperscript{-4} M</td>
</tr>
<tr>
<td>Na\textsubscript{2}CO\textsubscript{3}</td>
<td>---</td>
<td>4.03 g</td>
<td>3.80 x 10\textsuperscript{-5} M</td>
</tr>
<tr>
<td>K\textsubscript{2}HPO\textsubscript{4}</td>
<td>---</td>
<td>0.50 g</td>
<td>2.87 x 10\textsuperscript{-6} M</td>
</tr>
<tr>
<td><strong>Solution II</strong></td>
<td>500 mL</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>NaNO\textsubscript{3}</td>
<td>---</td>
<td>2.5 g</td>
<td>2.94 x 10\textsuperscript{-5} M</td>
</tr>
<tr>
<td>K\textsubscript{2}SO\textsubscript{4}</td>
<td>---</td>
<td>1.0 g</td>
<td>5.74 x 10\textsuperscript{-6} M</td>
</tr>
<tr>
<td>NaCl</td>
<td>---</td>
<td>1.0 g</td>
<td>1.71 x 10\textsuperscript{-5} M</td>
</tr>
<tr>
<td>MgSO\textsubscript{4} 7H\textsubscript{2}O</td>
<td>---</td>
<td>0.2 g</td>
<td>8.11 x 10\textsuperscript{-7} M</td>
</tr>
<tr>
<td>CaCl\textsubscript{2} 2H\textsubscript{2}O</td>
<td>---</td>
<td>0.04 g</td>
<td>2.72 x 10\textsuperscript{-7} M</td>
</tr>
<tr>
<td>FeSO\textsubscript{4} 7H\textsubscript{2}O</td>
<td>---</td>
<td>0.01 g</td>
<td>3.60 x 10\textsuperscript{-8} M</td>
</tr>
<tr>
<td>Na\textsubscript{2}EDTA 2H\textsubscript{2}O</td>
<td>---</td>
<td>0.08 g</td>
<td>2.15 x 10\textsuperscript{-7} M</td>
</tr>
<tr>
<td>trace metals solution</td>
<td>(see recipe below)</td>
<td>1 mL</td>
<td>---</td>
</tr>
<tr>
<td>vitamin stock solution</td>
<td>(see recipe below)</td>
<td>1 mL</td>
<td>---</td>
</tr>
</tbody>
</table>
Trace Metals Solution

Begin with 900 mL of dH₂O and dissolve the EDTA. Independently, dissolve each component and bring final volume to 1 liter. Schlösser (1994) recommends making two trace metals solutions, (a) 0.4 g EDTA and 0.7 g FeSO₄ 7H₂O in 100 mL dH₂O, and (b) 0.4 g EDTA and remaining elements in 900 mL dH₂O; autoclave separately and combine aseptically when cool.

<table>
<thead>
<tr>
<th>Component</th>
<th>Primary Stock Solution</th>
<th>Quantity</th>
<th>Molar Concentration in Final Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na₂EDTA 2H₂O</td>
<td>---</td>
<td>0.8 g</td>
<td>2.15 x 10⁻⁶ M</td>
</tr>
<tr>
<td>FeSO₄ 7H₂O</td>
<td>---</td>
<td>0.7 g</td>
<td>2.52 x 10⁻⁶ M</td>
</tr>
<tr>
<td>ZnSO₄ 7H₂O</td>
<td>1.0 g L⁻¹ dH₂O</td>
<td>1 mL</td>
<td>3.48 x 10⁻⁹ M</td>
</tr>
<tr>
<td>MnSO₄ 7H₂O</td>
<td>2.0 g L⁻¹ dH₂O</td>
<td>1 mL</td>
<td>8.97 x 10⁻⁹ M</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>10.0 g L⁻¹ dH₂O</td>
<td>1 mL</td>
<td>1.62 x 10⁻⁷ M</td>
</tr>
<tr>
<td>Co(NO₃)₂ 6H₂O</td>
<td>1.0 g L⁻¹ dH₂O</td>
<td>1 mL</td>
<td>3.44 x 10⁻⁹ M</td>
</tr>
<tr>
<td>Na₂MoO₄ 2H₂O</td>
<td>1.0 g L⁻¹ dH₂O</td>
<td>1 mL</td>
<td>4.13 x 10⁻⁹ M</td>
</tr>
<tr>
<td>CuSO₄ 5H₂O</td>
<td>0.005 g L⁻¹ dH₂O</td>
<td>1 mL</td>
<td>2.00 x 10⁻¹¹ M</td>
</tr>
</tbody>
</table>

Cyanocobalim Stock Solution

Dissolve the cyanocobalamin in 1 liter of dH₂O and filter sterilize. Store frozen.

<table>
<thead>
<tr>
<th>Component</th>
<th>Primary Stock Solution</th>
<th>Quantity</th>
<th>Molar Concentration in Final Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>cyanocobalamin (vit. B₁₂)</td>
<td>---</td>
<td>5 mg</td>
<td>3.69 x 10⁻⁹ M</td>
</tr>
</tbody>
</table>